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IN REPLY, REFER TO
DEPARTMENT OF PATHOLOGY

TELEPHONE,
(213) 226-2191

July 7, 1975

Vincent F. Lisanti, D.M.D.
The Council for Tobacco Research - USA
110 East 59th Street
New York, New York 10022

Dear Dr. Lisanti:

As I said in my letter of July 3rd, I am sending photos of post-influenzal lesions in C57Bl/6J vitamin A deficient mice. This is the first vitamin A experiment in C57Bl mice carried out in the laboratory. They are (Plates I and II) taken from slides sent you for Dr. Sommers' inspection several days ago. These are extremely spectacular, in my opinion, in that the lesions resemble very much those which Nettesheim et al (J. Nat. Cancer Inst. 47: 697-701, 1971) reported in mice following the intra-nasal instillation of 3-methylcholanthrene (MCA). A second experiment is in progress during which the post-influenzal lesions will be observed for a longer period of time and transplants will be made in vitamin A deficient and non-deficient mice.

The post-influenzal hyalinized lesions also resemble those reported by Davis et al (Brit. J. Cancer 31, 1975) in rats following intratracheal instillation of 3-4Benzpyrene (Figs. 2,3,4,5,6 on pp. 448-51), from repeated instillations of tobacco smoke condensates (Fig. 3, p.460), and from inhaled tobacco smoke with or without exposure to 3-4 benzpyrene (Figs. 4&5, p. 474). I am more and more convinced that there is an infectious component to these experimental tumors and that interference with vitamin A function probably plays a role. The studies which we have outlined for the coming year will help answer these questions.

With further reference to the studies of Davis et al, I am not convinced that their interpretation that the alveolar epithelium undergoes cuboidal cell metaplasia is correct. Our studies with Sendai virus infections show that

alveolar epithelialization occurs as a result of peripheral growth of bronchial lining membranes which replace the regular alveolar linings. These can be shown by employing G1-6-PDH and LDH enzyme staining procedures which differentiate cells derived from bronchial and alveolar epithelium respectively.

We are beginning to study, by electron microscopy and other means, pulmonary macrophages in the lungs of animals subjected to cigarette smoke inhalation. In a previous report given at the 31st Annual Meeting of the Electron Microscopy Society of America, 1973, crystals in pulmonary macrophages in old mice (CD-1 strain), living in synthetic smog and filtered air, were reported (see enclosed reprint). It was of interest that cytoplasmic crystals were not seen in pulmonary macrophages from animals sacrificed before 12 months of age.

Likewise, cytoplasmic crystals have not been seen in pulmonary macrophages up to 12 months of age in C57Bl/6J, C57L/J and SWR/J mice. Animals sacrificed after this time show varying numbers of crystals and other inclusions in pulmonary macrophages in shelf control, sham control, and smoke exposed mice (C57Bl/6J Plates III to VII), C57L/J (Plates VIII to XIII), and SWR/J (Plates XIV to XVIII). Macrophages containing crystals and other cytoplasmic inclusions were also observed in the peribronchial interstitial tissue of the smoke exposed mice. The cytoplasmic crystals and other inclusions in pulmonary macrophages of smoke exposed mice resemble those recently reported by Brody and Craighead (Lab. Invest. 32(2): 125-132, 1975.)

We have the opportunity to study the nature of the cytoplasmic crystals and amorphous debrae with energy-dispersive X-ray spectrometry similar to that employed by Brody and Craighead. Dr. Taylor, Associate Professor of Pathology and Chief of E.M. and X-ray spectrometry of the Coroner's Office at the LAC-USC Medical Center, will collaborate in carrying out these studies. His laboratory is equipped with apparatus similar to that used by Brody and Craighead. In addition, with the above procedures, we also plan to analyze whole tobacco and cigarette paper, ash from burned cigarettes, and smoke particles collected on cambridge filters.

What is of interest to us is that intracytoplasmic crystals are a characteristic finding in pulmonary macrophages from old animals whether or not they have been subjected to cigarette smoke inhalation.

As was stated in the progress report VII, pulmonary adenomas were not seen in the three groups of mice sacrificed after cigarette smoke exposure for 12 months. However, some were present in the lungs of the SWR/J mice sacrificed after 19 months of smoke inhalation (June 17th, 1975). They were present in about the same frequency (30 to 40 percent) in the lungs of shelf control, sham control, and smoke exposed vitamin A deficient and non-deficient groups. This indicates to us that the appearance of the pulmonary adenomas is related to the age of the animals and not to cigarette smoke inhalation. A more detailed analysis of the SWR/J lungs is being made. These findings also show the importance of continuing the smoke exposure of the C57Bl/6J and C57L/J mice for another several months.

Sincerely,

Clayton G. Loosli, M.D.
Hastings Professor of
Medicine and Pathology

CGL:ra

Enclosures